CLAIMS

1. An isolated nucleic acid fragment which

- encodes a polypeptide fragment which exhibits a substantial immunological reactivity with a rabbit polyclonal antibody raised against a polypeptide having an apparent molecular weight of 13 kDa as determined by SDS-PAGE followed by visualization, said polypeptide being derived from *Borrelia burgdorferi* B313 and being encoded by the nucleotide sequence of SEQ ID NO: 18, said rabbit polyclonal antibody exhibiting substantially no immunological reactivity with proteins from at least 95% of spirochaetes randomly selected from the group consisting of *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*, and/or

- hybridises readily under highly stringent hybridization conditions with a DNA fragment having a nucleotide sequence selected from the group consising of SEQ ID NO: 18, SEQ ID NO: 20, and SEQ ID NO: 22, or with a DNA fragment complementary thereto, but exhibits no substantial hybridization when the hybridization conditions are highly stringent with genomic DNA from at least 95% of spirochaetes randomly selected from the group consisting of *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

2. The nucleic acid fragment according to claim 1, which encodes a polypeptide fragment comprising an amino acid sequence comprised in a polypeptide, said polypeptide being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, and/or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, or *Borrelia hispanica*.

3. The nucleic acid fragment according to claim 1 or 2, which encodes a polypeptide fragment comprising at least a part of an amino acid sequence of a protein having an apparent molecular weight of 13 kDa, said protein being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, and/or *Borrelia afzelii* ACAI but being substantially absent from whole cell

preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

4. The nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which encodes a polypeptide fragment comprising at least one epitope, said epitope being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

5. The nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which encodes a polypeptide fragment comprising at least one epitope of a protein having an apparent molecular weight of 13 kDa, said protein being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

6. The nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which encodes a polypeptide fragment which has an amino acid sequence exhibiting a sequence identity of at least 50% with SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23, or with subsequences thereof having a length of at least 10 amino acid residues.

7. The nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, wherein the nucleotide sequence has a sequence homology of at least 70% with SEQ ID NO: 18, SEQ ID NO: 20, or SEQ ID NO: 22, or with subsequences thereof having a length of at least 12 nucleotides.

8. A nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which comprises a nucleic acid fragment encoding a polypeptide fragment which comprises at least one amino acid sequence selected from the group consisting of amino acid residues 19-27, 33-36, 41-47, 95-104, 138-147 and 174-179 in SEQ ID NO: 19; amino acid residues 19-26, 32-35, 40-47, 94-101, 137-146, and 174-178 in SEQ ID NO: 21; and amino acid residues 18-26, 30-33, 39-46, 91-104, 137-145 and 173-177 in SEQ ID NO: 23.

9. The nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which encodes a protein having an apparent molecular weight of 13 kDa which is present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but which is substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

10. The nucleic acid fragment according to claim 9, wherein the encoded protein is present in fraction B from *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI.

11. The nucleic acid fragment according to claim 10, wherein the encoded protein is a surface exposed protein of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI.

12. A nucleic acid fragment according to ~~any of the preceding claims~~ claim 1 which comprises a nucleotide sequence encoding a polypeptide fragment which includes an amino acid seqeuence selected from the group consisting of SEQ ID NOs: 19, 21, and 23.

13. A nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 18, 20, and 22.

14. A nucleic acid fragment according to claim 12 which consists of a nucleotide sequence encoding a polypeptide fragment consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 21, and 23.

15. A nucleic acid fragment according to claim 13, which consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 18, 20, and 22.

16. A nucleic acid fragment according to ~~any of claims 1-13~~ claim 1, which encodes a fusion polypeptide.

17. The nucleic acid fragment according to claim 16, which encodes a fusion polypeptide which comprises, as a fusion partner, a polypeptide fragment which enhances the immunogenicity of the fusion polypeptide relative to the immunogenicity of a polypeptide not comprising said second fusion partner or which facilitates the expression of the fusion polypeptide in a host cell and/or the subsequent purification of the polypeptide.

18. The nucleic acid fragment according to claim 16 ~~or 17~~, which encodes a fusion polypeptide comprising, as a fusion partner, a polypeptide fragment which

- has the same amino acid sequence as at least one amino acid sequence selected from the group consisting of amino acid residues 19-27, 33-36, 41-47, 95-104, 138-147 and 174-179 in SEQ ID NO: 19; amino acid residues 19-26, 32-35, 40-47, 94-101, 137-146, and 174-178 in SEQ ID NO: 21; and and amino acid residues 18-26, 30-33, 39-46, 91-104, 137-145 and 173-177 in SEQ ID NO: 23,

- is a lipoprotein selected from the outer membrane lipoprotein from *E. coli* and OspA from *Borrelia burgdorferi sensu lato*,

- is a viral protein selected from Hepatitis B surface antigen, Hepatitis B core antigen, and the influenza virus non-structural protein NS1,

- is an immunoglobulin binding protein selected from protein A, protein G, and the ZZ-peptide,

- is a T-cell epitope,

- is a B-cell epitope,

- is a bacterial fimbrial protein selected from the pilus components pilin and papA, and/or

- is the maltose binding protein, glutathione S-transferase, β-galactosidase, calmodulin binding protein or poly-histidine.

19. The nucleic acid fragment according to ~~any of the preceding claims~~ claim 1, which is a DNA fragment.

20. A polypeptide fragment which exhibits a substantial immunological reactivity with a polyclonal rabbit antibody raised against a polypeptide having an apparent molecular weight of 13 kDa as determined by SDS PAGE followed by visualization and being derived from *Borrelia burgdorferi* B313, said polypeptide comprising the amino acid sequence 1-167 of SEQ ID NO: 19, said polyclonal rabbit antibody exhibiting substantially no immunological reactivity with whole cell preparations from at least 95% of randomly selected *B. hermsii*, *B. crocidurae*, *B. anserina*, or *B. hispanica*, with the proviso that said polypeptide is essentially free from other *Borrelia*-derived antigens when it is identical in amino acid sequence to a 13 kDa surface exposed polypeptide which can be extracted from *Borrelia burgdorferi sensu lato*,

the polypeptide fragment optionally being lipidated.

21. The polypeptide fragment according to claim 20, which comprises an amino acid sequence comprised in a polypeptide, said polypeptide being present in whole cell preparations of *Borrelia burgdorferi* B31, ~~*Borrelia burgdorferi* B313~~, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, or *Borrelia hispanica*.

22. The polypeptide fragment according claim 20 ~~or 21~~, which comprises at least a part of the amino acid sequence of a protein having an apparent molecular weight of 13 kDa, said protein being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

23. A polypeptide fragment according to ~~any of claims 20-22~~, which comprises at least one epitope, said epitope being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

24. A polypeptide fragment according to ~~any of claims 20-23~~, which comprises at least one epitope of a protein having an apparent molecular weight of 13 kDa, said protein being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI but being substantially absent from whole cell preparations of at least 95% of randomly selected *Borrelia hermsii*, *Borrelia crocidurae*, *Borrelia anserina*, and *Borrelia hispanica*.

25. The polypeptide fragment according to ~~claim any of claims 20-24~~, which comprises at least one amino acid sequence selected from the group consisting of amino acid residues 19-27, 33-36, 41-47, 95-104, 138-147 and 174-179 in SEQ ID NO: 19; amino acid residues 19-26, 32-35, 40-47, 94-101, 137-146, and 174-178 in SEQ ID NO: 21; and amino acid residues 18-26, 30-33, 39-46, 91-104, 137-145 and 173-177 in SEQ ID NO: 23.

26. The polypeptide fragment according to ~~any of claims 20-25~~, which has an amino acid sequence identical to that of a protein having an apparent molecular weight of 13 kDa and being present in whole cell preparations of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI.

27. The polypeptide fragment according to claim 26, wherein the protein is present in fraction B from *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI.

28. The polypeptide fragment according to claim 26 ~~or 27~~, wherein the protein is a surface exposed protein of *Borrelia burgdorferi* B31, *Borrelia burgdorferi* B313, *Borrelia garinii* IP90, or *Borrelia afzelii* ACAI.

29. The polypeptide fragment according to ~~any of claims 20-28~~ claim 20; which has an amino acid sequence exhibiting a sequence identity of at least 50% with an amino acid sequence selected from SEQ ID NOs: 19, 21, and 23, or with a subsequence thereof of at least 10 amino acids.

30. A polypeptide fragment according to ~~any of claims 20-29~~ claim 20, which is encoded by a nucleotide sequence exhibiting a sequence identity of at least 70% with a sequence selected from the group consisting of SEQ ID NOs: 18, 20, and 22, or with a subsequence thereof of at least 12 nucleotides.

31. The polypeptide fragment according to claim 29, which comprises an amino acid sequence selected from SEQ ID NOs: 19, 21, and 23.

32. The polypeptide fragment according to claim 31, which is encoded by a DNA fragment comprising a nucleotide sequence selected from SEQ ID NOs: 18, 20, and 22.

33. A fusion polypeptide comprising as a first fusion partner the polypeptide according to ~~any of claims 20-32~~ claim 20.

34. The fusion polypeptide according to claim 33, which comprises, as a second fusion partner, a polypeptide fragment which enhances the immunogenicity of the fusion polypeptide relative to the immunogenicity of a polypeptide not comprising said second fusion partner or which facilitates the expression of the fusion polypeptide in a host cell and/or the subsequent purification of the polypeptide.

35. The fusion polypeptide according to claim 33 or 34, wherein at least one second fusion partner is a polypeptide

- which has the same amino acid sequence as at least one amino acid sequence selected from the group consisting of amino acid residues 19-27, 33-36, 41-47, 95-104, 138-147 and 174-179 in SEQ ID NO: 19; amino acid residues 19-26, 32-35, 40-47, 94-101, 137-146, and 174-178 in SEQ ID NO: 21; and amino acid residues 18-26, 30-33, 39-46, 91-104, 137-145 and 173-177 in SEQ ID NO: 23,

- which is a lipoprotein selected from the outer membrane lipoprotein from *E. coli* and OspA from *Borrelia burgdorferi sensu lato*,

- which is a viral protein selected from Hepatitis B surface antigen, Hepatitis B core antigen, and the influenza virus non-structural protein NS1,

- which is an immunoglobulin binding protein selected from protein A, protein G, and the synthetic ZZ-peptide,

- which is a T-cell epitope,

- which is a B-cell epitope,

- which is a bacterial fimbrial protein selected from the pilus components pilin and papA, and/or

- which is the maltose binding protein, glutathione S-transferase, β-galactosidase, or poly-histidine.

36. A non-borrelial vector carrying the nucleic acid fragment according to ~~any of claims 1-19~~ claim 1.

37. The vector according to claim 36, which is capable of autonomous replication.

38. The vector according to claim 36, which is selected from the group consisting of a plasmid, a phage, a cosmid, a mini-chromosome, and a virus.

39. A vector according to ~~any of claims 36-38~~ claim 36, which, when introduced in a host cell, is integrated in the host cell genome.

40. A vector according to any of claims 36-39, wherein the vector comprises, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to any of claims 1-19, a nucleic acid sequence

encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to any of claims 1-19, and a nucleic acid sequence encoding a terminator.

41. A vector according to ~~claim~~ 40, wherein the promoter drives expression in a eukaryotic cell.

42. A vector according to claim 40 ~~or 41~~, wherein the leader peptide enables secretion from or integration into the membrane of a mammalian cell.

43. A transformed cell carrying the vector of any of claims 36-42 and capable of replicating the nucleic acid fragment according to any of claims 1-19.

44. A transformed cell according to claim 43, which is a microorganism selected from a bacterium, a yeast, a protozoan, or a cell derived from a multicellular organism selected from a fungus, an insect cell, a plant cell, and a mammalian cell.

45. A transformed cell according to claim 44 which is a bacterium of the genus *Escherichia*, *Bacillus* or *Salmonella*.

46. A transformed cell according to claim 45, which is an *E. coli* cell.

47. A stable cell line producing the polypeptide according to any of claims 20-35, which carries the vector according to any of claims 36-42 and which expresses the nucleic acid fragment according to any of claims 1-19.

48. A method of preparing a polypeptide fragment as defined in any of claims 1-19, the method comprising

- culturing the transformed cell according to any of claims 43-46 or the stable cell line according to claim 47 under conditions facilitating the expression of the polypeptide fragment thereby, and

- harvesting the polypeptide fragment, and optionally subjecting the polypeptide to post-translational modification(s);

or

- synthesising the polypeptide fragment by solid-phase peptide synthesis or by liquid-phase peptide synthesis.

49. A method according to claim 48, wherein the post-translational modifications involve lipidation, glycosylation, cleavage and/or elongation.

50. A vaccine comprising an amount of the polypeptide fragment according to ~~any of claims 20-35 or of the polypeptide fragment prepared by the method according to claim 48 or 49~~, the amount of the polypeptide fragment being effective to confer substantially increased resistance to infections with *Borrelia burgdorferi sensu lato* in an animal, including a human being, the polypeptide fragment being formulated in combination with a pharmaceutically acceptable carrier, diluent or vehicle, and the vaccine optionally further comprising an adjuvant.

51. A vaccine according to claim 50, wherein the pharmaceutically acceptable carrier, vehicle, or diluent is selected from the group consisting of sterile water, physiological saline, glucose, polyalkalene glycols, and triglycerides; and wherein the adjuvant is selected from the group consisting of aluminium hydroxide or phosphate (alum), synthetic polymers of sugars (Carbopol), bacterial cells such as *C. parvum* or endotoxins or lipopolysaccharide components of gram-negative bacteria, physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A), a perfluorocarbon (Fluosol-DA).

52. A vaccine according to ~~claim 50 or 51~~, wherein the amount of the polypeptide fragment is in the range of 1-1000 µg per dose unit, such as between 2 and 750 µg, between 5 and 500 µg, between 7.5 and 250 µg, between 10 and 150 µg, between 10 and 100 µg, between 10 and 75 µg, and between 10 and 50 µg.

53. A live vaccine comprising a non-pathogenic microorganism carrying and being capable of expressing the nucleic acid fragment according to any of claims 1-19 so as to produce the polypeptide fragment according to any of claims 20-35, the live vaccine being effective in conferring increased resistance to infection with *Borrelia burgdorferi sensu lato* in an animal, including a human being.

54. The live vaccine according to claim 53, wherein the non-pathogenic microorganism is selected from the group consisting of *Mycobacterium bovis* BCG, *Salmonella typhi, Salmonella typhimurium, Salmonella paratyphi, Staphylococcus aureus*, and *Listeria monocytogenes*.

55. A combination vaccine comprising

an amount of the polypeptide fragment according to ~~any of claims 20-35 or of the polypeptide fragment prepared by the method according to claim 48 or 49~~ claim 20, the amount of the polypeptide fragment being effective to confer substantially increased resistance to infections with *Borrelia burgdorferi sensu lato* in an animal, including a human being;

and

at least one further Borrelia antigen,

the polypeptide fragment and the antigen being formulated in combination with a pharmaceutically acceptable carrier, vehicle, or diluent and the vaccine optionally further comprising an adjuvant.

56. A combination vaccine according to claim 55, wherein the at least one further Borrelia antigen is selected from the group consisting of OspA, OspB, OspC, OspD, OspE, OspF, OspG, PC, Oms28, Oms45, Oms 66, decorin binding protein (dbp), LpLA7, EppA, T5, S1, 26 kDa, 39 kDa, 66 kDa, 79 kDa, 85 kDa, and 110 kDa antigen.

57. A combination vaccine comprising at least two non-identical polypeptide fragments according to ~~any of claims 20-35 or at least two non-identical polypeptide fragments prepared by the method according to claim 48 or 49~~, the vaccine comprising an amount of the polypeptide fragments effective to confer substantially increased resistance to infections with *Borrelia burgdorferi sensu lato* in an animal, including a human being, in combination with a pharmaceutically acceptable carrier, vehicle, or diluent, the vaccine optionally further comprising an adjuvant.

58. A vaccine comprising the nucleic acid fragment according to any of claims 1-19 or a vector according to any of claims 36-42, the vaccine effecting *in vivo* expression of antigens by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed antigens being effective to confer substantially increased resistance to infections with *Borrelia burgdorferi sensu lato* in an animal, including a human being.

59. A diagnostic composition adapted for the determination of *Borrelia burgdorferi sensu lato* in a sample, the composition comprising the polypeptide fragment according to ~~any of claims 20-35 or the polypeptide fragment prepared by the method according to claim 48 or 49~~, the amount of the polypeptide fragment being effective to detectably react with antibodies present in the sample, the antibodies being directed against *Borrelia burgdorferi sensu lato*, the composition optionally comprising a detectable label.

60. A diagnostic composition adapted for the determination of *Borrelia burgdorferi sensu lato* in a sample, the composition comprising an amount of the nucleic acid fragment according to ~~any of claims 1-19~~ which is effective to detectably bind to a nucleic acid fragment from *Borrelia burgdorferi sensu lato* present in the sample, the composition optionally comprising a detectable label.

61. A method of immunizing an animal, including a human being, against infections with *Borrelia burgdorferi sensu lato*, the method comprising administering to the animal an immunogenically effective amount of the vaccine according to ~~any of claims 50-58~~.

62. A method of determining the presence of antibodies directed against *Borrelia burgdorferi sensu lato* in a sample, comprising incubating the sample with the polypeptide fragment according to ~~any of claims 20-35 or with the polypeptide fragment prepared by the method according to claim 48 or 49,~~ and detecting the presence of bound antibody resulting from the administration or incubation.

63. A method of determining the presence of *Borrelia burgdorferi sensu lato* nucleic acids in a sample, comprising incubating the sample with the nucleic acid fragment according to ~~any of claims 1-19~~, and detecting the presence of hybridized nucleic acids resulting from the incubation.

64. A method for determining the presence of *Borrelia burgdorferi sensu lato* nucleic acids in a sample, comprising subjecting the nucleic acid fragment according to ~~any of claims 1-19~~ to a molecular amplification reaction, such as PCR, and detecting the presence of amplified nucleic acid which is specific for *Borrelia burgdorferi sensu lato*.

65. A diagnostic kit comprising

- a polypeptide fragment according to any of claims 20-35 and a means for detecting the the polypeptide fragment with antibody bound thereto,

- a nucleic acid fragment according to any of claims 1-19 and a means for detecting the binding between the nucleic acid fragment and nucleic acid bound thereto, or

- a set of nucleic acid primers which, when used in a molecular amplification procedure together with the nucleic acid fragment according to any of claims 1-19, will result in specific amplification of said nucleic acid fragment, and a means for detecting the amplified nucleic acid fragment.

66. A method for the preparation of an immunological composition such as a vaccine, the method comprising the steps of admixing

- a polypeptide fragment according to ~~any of claims 20-35 or prepared according to the method of claim 48 or 49~~,

- a pharmaceutically acceptable carrier, vehicle, or diluent, and optionally

- an adjuvant.